

## Invited review

## Ryanodine receptors as pharmacological targets for heart disease<sup>1</sup>

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### Key words

arrhythmias; calcium release channel; heart failure; pharmacology; ryanodine receptor

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### Abstract

Calcium release from intracellular stores plays an important role in the regulation of muscle contraction and electrical signals that determine the heart rhythm. The ryanodine receptor (RyR) is the major calcium (Ca<sup>2+</sup>) release channel required for excitation-contraction coupling in the heart. Recent studies have demonstrated that RyR are macromolecular complexes comprising of 4 pore-forming channel subunits, each of which is associated with regulatory subunits. Clinical and experimental studies over the past 5 years have provided compelling evidence that intracellular Ca<sup>2+</sup> release channels play a pivotal role in the development of cardiac arrhythmias and heart failure. Changes in the channel regulation and subunit composition are believed to cause diastolic calcium leakage from the sarcoplasmic reticulum, which could trigger arrhythmias and weaken cardiac contractility. Therefore, cardiac RyR have emerged as potential therapeutic targets for the treatment of heart disease. Consequently, there is a strong desire to identify and/or develop novel pharmacological agents that may target these Ca<sup>2+</sup> signaling pathways. Pharmacological agents known to modulate RyR in the heart, and their potential application towards the treatment of heart disease are discussed in this review.

### Introduction

Fluctuations in cytosolic calcium (Ca<sup>2+</sup>) concentrations act to modulate a vast array of physiological processes, including neurotransmitter release, cell division, and muscle contraction. This control mechanism requires low cytosolic Ca<sup>2+</sup> levels under resting conditions alternating with transient increases in Ca<sup>2+</sup> upon activation. In cardiac muscle, transverse tubular invaginations of the plasma membrane contact the membrane of the sarcoplasmic reticulum (SR) Ca<sup>2+</sup> stores to form dyadic junctions, establishing a structural framework for the external regulation of intracellular Ca<sup>2+</sup> release<sup>[1]</sup>. Two principal classes of intracellular ion channels have evolved to facilitate the movement of Ca<sup>2+</sup> into the cytosol from intracellular stores: (i) inositol 1,4,5-trisphosphate receptors; and (ii) ryanodine receptors (RyR)<sup>[2]</sup>. This review article will focus on cardiac (type 2) RyR (RyR2), as increasing evidence emerges that RyR play a pivotal role in the development of cardiac arrhythmias and heart failure. We will discuss classic and novel pharmacological agents

that may modulate these calcium release channels in order to treat cardiac disease.

### Ryanodine receptors (RyR)

RyR consist of large tetramers of RyR monomers, comprised of a large regulatory domain protruding into the cytosol and a much smaller transmembrane domain containing the channel pore<sup>[3]</sup>. It is now well accepted that RyR2 channels exist as large macromolecular complexes comprised of numerous regulatory subunits, including calmodulin (CaM), the FK506-binding protein FKBP12.6 (also known as calstabin2), protein kinase A (PKA), CaM-dependent kinase II, protein phosphatases 1 and 2A, phosphodiesterase, junctin, triadin, and calsequestrin<sup>[4]</sup>. The gating behavior of RyR can be regulated by many of these accessory proteins (CaM, FKBP12.6, and PKA) as well as a variety of endogenous ligands (Ca<sup>2+</sup>, ATP, Mg<sup>2+</sup>)<sup>[5]</sup>. The physical and functional association of RyR2 channels results in coordinated gating behavior termed "coupled gating"<sup>[6]</sup>. Coupled gating

requires FKBP12.6 in the RyR2 macromolecular complex, and allows clusters of channels to function as  $\text{Ca}^{2+}$  release units that release calcium amounts that can be visualized as  $\text{Ca}^{2+}$  sparks<sup>[7]</sup>. As the functional effects of the RyR2 accessory subunits have been reviewed previously, this will not be the focus of the present article<sup>[4,8]</sup>.

The dysfunction of RyR2 has been implicated in various diseases of the heart. A number of inherited mutations in the *RyR2* gene have been identified in patients with exercise-induced ventricular arrhythmias and sudden cardiac death<sup>[9-11]</sup>. Although initially most mutations were identified in the amino terminus, central domain, and carboxy terminus, more recent genetic data suggest that mutations may occur throughout the channel protein<sup>[12]</sup>. In addition to these relatively rare genetic arrhythmias, acquired RyR2 defects have been implicated in the development of congestive heart failure<sup>[13,14]</sup>. Clinical and experimental data suggest that in failing hearts, the phosphorylation status of RyR2 is altered due to chronic hyperactivity of PKA<sup>[4,8,15]</sup>. The hyperphosphorylation of RyR2 by PKA is associated with the loss of the channel-stabilizing subunit FKBP12.6, which alters the activation properties of RyR2 and increases the open probability<sup>[13,16]</sup>. Moreover, it has been suggested that coupled gating between RyR2 may be altered in the failing heart, which might decrease systolic  $\text{Ca}^{2+}$  transients and/or cause a diastolic  $\text{Ca}^{2+}$  leak<sup>[4,6]</sup>. The unwanted diastolic leak of  $\text{Ca}^{2+}$  from the SR promotes the generation of arrhythmias and anomalous contraction of the heart. Although some of the mechanistic aspects of this concept have been debated in recent articles, most authors agree that a diastolic SR  $\text{Ca}^{2+}$  leak promotes arrhythmias and heart failure<sup>[17,18]</sup>. Therefore, RyR have emerged as novel therapeutic targets for the treatment of inherited arrhythmias and heart failure<sup>[19]</sup>.

## RyR pharmacology

The activity of RyR is regulated by multiple endogenous

proteins residing in the macromolecular channel complex as indicated earlier. The focus of this review, however, will be on exogenous pharmacological agents that have been shown to interact with and modulate cardiac RyR<sup>[20]</sup>. These agents may be classified according to their effect on the SR  $\text{Ca}^{2+}$  release function (eg agonist or antagonist), or according to the mechanism of RyR2 modulation (Tables 1, 2). One class of agents modulates RyR2 primarily by altering gating of the channel (ie the opening and closing of the ion-conducting pathway), for example, by increasing the sensitivity to  $\text{Ca}^{2+}$ -induced activation of RyR2. A second group of molecules acts by controlling the movement of ions through the pore of the RyR2 channel, for example, by entering the pore and physically obstructing ion passage. A third group of compounds may alter RyR2 function by enhancing the interaction between subunits within the RyR2 macromolecular channel complex, or even between different RyR2 channel units (ie enhancing coupled gating). Although none of these compounds are currently used for the treatment of patients with heart failure or arrhythmias, the emphasis of this review will be on the potential therapeutic applications or non-therapeutic side effects of RyR2 modulating agents. Based on recent advances in our understanding of  $\text{Ca}^{2+}$ -handling defects in heart failure and cardiac arrhythmias, one could

**Table 1.** Classification of RyR2 modulating agents (activity based).

Agonists	Antagonists
Purine derivatives (caffeine)	Ruthenium red
Digitalis glycosides (digoxin)	Dantrolene
Suramin	Ryanoids (ryanodine)
Volatile anesthetics (halothane)	Local anesthetics (tetracaine)
4-CMC	1,4-Benzothiazepines
Peptide toxins (IpTx)	(JTV519, K201)
Macrocyclic compounds (FK506)	

**Table 2.** Classification of RyR2 modulating agents (mechanism based).

Modulators of channel gating	Modulators of ion translocation	Allosteric modulator subunit interactions
Purine derivatives (caffeine)	Ruthenium red	Macrocyclic compounds (FK506)
Digitalis glycosides (digoxin)	Ryanoids (ryanodine)	1,4-Benzothiazepines (JTV519, K201)
Suramin	Local anesthetics (tetracaine)	
Volatile anesthetics (halothane)		
4-CMC		
Peptide toxins (IpTx)		
Dantrolene		

profile an ideal drug for the modulation of RyR2. Such compounds would not interfere with systolic SR Ca<sup>2+</sup> release, as this would depress cardiac contractility. However, inhibition of a diastolic SR Ca<sup>2+</sup> leak would be desirable, as it is likely to prevent arrhythmias and enhances SR Ca<sup>2+</sup> loading, which could improve contractility.

## RyR2 agonists

**Purine derivatives and related compounds** This group includes substances that have a similar sterical structure based on a purine, carboline, carbazole, or imidazopyridine ring, and are likely to act on the same molecular site. Methylxanthine compounds, like caffeine and theophylline, isolated from the leaves and beans of certain plants, activate RyR2 by potentiating its sensitivity to the natural ligand Ca<sup>2+</sup>. RyR2 is activated by millimolar concentrations of caffeine, which causes a pronounced increase in the sensitivity of RyR2 to Ca<sup>2+</sup> such that the channels open at basal (diastolic) Ca<sup>2+</sup> levels<sup>[21]</sup>. At low caffeine concentrations, caffeine increases the open probability of the RyR2 channel by increasing the frequency of channel openings alone, whereas at higher concentrations, it results from an increase in both the open channel lifetime and the frequency of RyR2 openings<sup>[22]</sup>. Theophylline and other methylxanthines share the mode of action of caffeine<sup>[23]</sup>. Although these effects are readily observed in the experimental setting, it is unlikely that RyR2 modulation will be important in the therapeutic response to methylxanthines because their plasma concentration (eg about 55 micromolar for theophylline) is lower than the effective concentration range<sup>[20]</sup>. Further compounds have been proposed to act in a similar manner to caffeine. The imidazopyridine derivative, sulmazole, increases the duration and frequency of RyR2 openings. Whereas the EC<sub>50</sub> for RyR2 activation by caffeine is between 0.2–1 millimolar, sulmazole displays much greater potency (400 μmol)<sup>[24,25]</sup>.

**Digitalis glycosides** Digoxin is one of the cardiac glycosides, a closely-related group of drugs that have in common specific effects on the myocardium. These drugs are found in a number of plants; digoxin is extracted from the leaves of *Digitalis lanata*. At a therapeutic concentration (~1 nM), digoxin increases the open probability of RyR2 by decreasing the lifetime of the closed states of the channel<sup>[26]</sup>. Digoxin appears to sensitize RyR2, as channel gating itself is not modified at picomolar Ca<sup>2+</sup> concentrations. The activation of RyR2, which is clearly distinct from the canonical Na<sup>+</sup>/K<sup>+</sup>-ATPase inhibiting action, might contribute to the inotropic effects of digoxin and digitoxin. Such actions are

similar to those of caffeine and sulmazole, but digitalis glycosides do not affect the RyR1 isoform<sup>[27]</sup>. Owing to its strong effects on Na<sup>+</sup>/K<sup>+</sup>-ATPase, it is unlikely that digoxin will be used clinically to modulate RyR2 in the heart.

**Suramin** Suramin is a polysulphonated naphthylurea, originally developed for the treatment of trypanosomiasis and is also used as an anticancer agent. In single channel experiments, suramin (in micromolar concentrations) increases the open probability of sheep cardiac RyR2 channels by stabilizing the open conformational state<sup>[28]</sup>. Recently, it has been suggested that the complex changes in RyR2 activity may result from an interaction with CaM-binding sites<sup>[29]</sup>. Thus, the suramin-induced potentiation of Ca<sup>2+</sup> release through RyR2 may involve a relief of CaM-induced inhibition. It is unclear at present whether suramin has any beneficial effects in animal models of heart failure.

**Volatile anesthetics** Several halogenated compounds affect SR Ca<sup>2+</sup> release. The most extensively studied are volatile anesthetics such as halothane, and its isomer isoflurane. Halothane has been shown to increase SR Ca<sup>2+</sup> release at gas concentrations ranging from about 0.002% to 3.8% (v/v) in a Ca<sup>2+</sup>- and pH-dependent manner<sup>[30,31]</sup>. At a physiological pH of 7.4, halothane increases RyR2 activity at all Ca<sup>2+</sup> concentrations without affecting channel conductance<sup>[31,32]</sup>. Similar effects have been observed with isoflurane and enflurane (2.5%–4%). A reduction of the pH from 7.4 to 7.1 causes maximum channel activation to occur at much lower cytosolic Ca<sup>2+</sup> concentrations<sup>[31]</sup>. Since the interaction of volatile anesthetics with RyR2 occurs at doses lower than the minimum effective alveolar concentration (ie ~0.7% for halothane and ~1.1% for isoflurane), their effects on RyR2 may produce negative inotropic effects and transient vasoconstriction<sup>[33]</sup>. Negative inotropy may result from enhanced diastolic Ca<sup>2+</sup> release via RyR2, which reduces the levels of Ca<sup>2+</sup> in the SR available for the subsequent systolic Ca<sup>2+</sup> release. This in turn reduces the amplitude of the Ca<sup>2+</sup> transient and suppresses cardiomyocyte contractility.

**4-Chloro-m-cresol** The phenol derivative 4-chloro-m-cresol (4-CMC) has been shown to increase the open probability of RyR1 incorporated in planar lipid bilayers by increasing both open lifetimes and frequencies<sup>[34]</sup>. In contrast to caffeine, 4-CMC can modulate channel gating from both the luminal and cytosolic sides of the channel. Whereas there are myriad data concerning the actions of 4-CMC on RyR1, relatively little is known about its action on the cardiac RyR2 isoform. In cell lines expressing recombinant RyR2, 4-CMC has been shown to enhance intracellular Ca<sup>2+</sup> release<sup>[35]</sup>. Although 4-CMC may modulate RyR2, the significance of these pharmacological effects remains to be

further explored.

**Peptide toxins** Several peptide toxins isolated from scorpion venoms have been shown to alter RyR2 activity, which has raised the prospect that animal venom may represent a unique source of selective modulators of intracellular  $\text{Ca}^{2+}$  release channels<sup>[36]</sup>. Two peptides isolated from the scorpion *Pandinus imperator*, imperatoxin A (IpTxa) and imperatoxin I (IpTxi), are highly selective for RyR and show no obvious activity with regard to other ion channels or transporters<sup>[37]</sup>. IpTxa is a small peptide comprising of 33 amino acids with a molecular weight of approximately 4 kDa. It specifically increases open probability of the RyR1 and RyR3 isoforms by sensitizing these channels to cytosolic  $\text{Ca}^{2+}$ , but has little effect on RyR2. However, single channel experiments have revealed that IpTxa induces the occurrence of a subconductance state equivalent to ~30% of the full conductance in all RyR isoforms, even though IpTxa has no effect on RyR2 in ryanodine binding assays<sup>[38]</sup>. IpTxi is a larger heterodimeric protein (~15 kDa) that consists of a large subunit comprising of 104 amino acids covalently linked via a disulfide bond to a smaller subunit of 27 amino acids<sup>[39]</sup>. Single channel studies have demonstrated that IpTxi inhibits both RyR1 and RyR2 with nanomolar affinity, although these effects may be mediated via a lipid product of its inherent phospholipase-2 (PLA-2) activity<sup>[39]</sup>. In spite of these elegant electrophysiological data, *in vivo* pharmacological experiments are needed to determine whether IpTx can improve cardiac contractility in animals with heart failure.

**Macrocyclic compounds** The macrolide immunosuppressant FK-506, also known as tacrolimus, can induce the dissociation of FKBP12.6 from RyR2, thereby altering RyR2 gating. Rapamycin is another macrolide immunosuppressant that can dissociate FKBP12.6 from the RyR2 channel complex. In cardiac muscle, 0.1–10  $\mu\text{mol/L}$  rapamycin increases single channel open probability and decreases channel conductance<sup>[40]</sup>. It has been speculated that the former effect is the consequence of drug binding to FKBP12.6, whereas the changes in channel conductance are the consequence of FKBP12.6 dissociation from RyR2<sup>[6,40]</sup>. Interference with the RyR2 subunit composition might be involved in some effects of FK506, particularly in the development of myocardial hypertrophy and heart failure, which has been observed in some pediatric transplant patients<sup>[41]</sup>.

## RyR2 antagonists

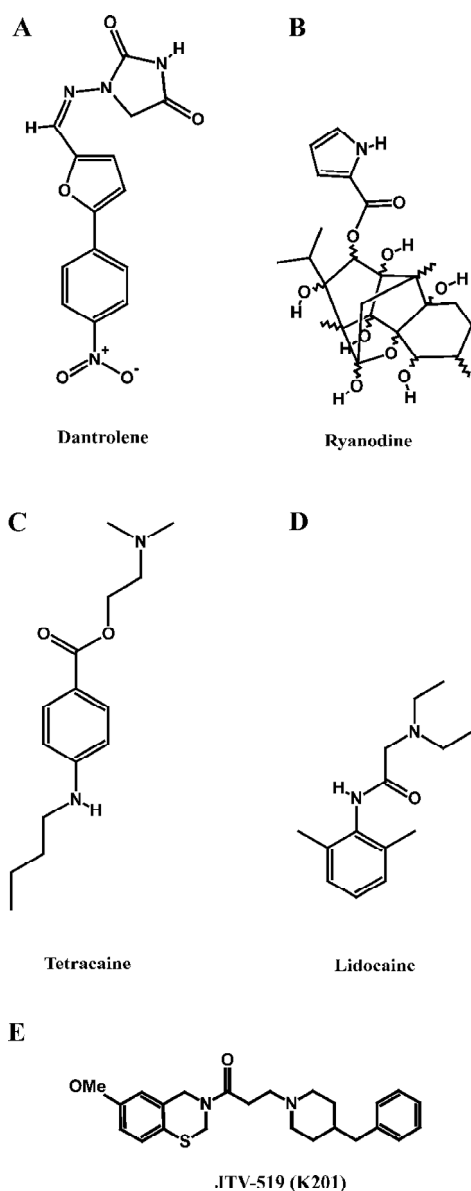
**Ruthenium red** Ruthenium red, a water-soluble dye with a structure that includes 14 amino groups, has been shown to inhibit SR  $\text{Ca}^{2+}$  release in cardiac muscle. In planar lipid

bilayer experiments, micromolar concentrations of ruthenium red dramatically decreases RyR2 open probability, associated with long-term channel closure<sup>[42]</sup>. At submicromolar concentrations, the major effect of ruthenium red is a decrease in the lifetime of the open channel state, whereas at higher concentrations (>1  $\mu\text{mol/L}$ ), the lifetime of the closed channel is increased. Ruthenium red reduces the RyR2 single channel current from both the cytosolic and luminal sides of the channel. However, the dwell times of the block are longer when ruthenium red is added to the luminal side. In addition, luminal ruthenium red decreases the single channel current without affecting channel open probability<sup>[43]</sup>. Binding studies performed using recombinant RyR1 channels have localized ruthenium red binding sites at residues 1861–2094 and 3657–3776<sup>[44]</sup>. On the basis of single channel studies, it has been proposed that the drug-binding site is located within the transmembrane domain, probably close to the channel pore region, and ruthenium red cannot permeate through the open channel<sup>[43]</sup>. Because ruthenium red is neurotoxic, it is not an ideal candidate for drug development.

**Dantrolene** Dantrolene is a hydantoin derivative commonly used for the treatment of the genetic disorder, malignant hyperthermia, which is caused by inherited mutations in RyR1 (Figure 1A). Importantly, dantrolene represents the only drug targeting RyR that is currently approved for clinical use. In skeletal muscle, 10–100 micromolar dantrolene inhibits abnormal  $\text{Ca}^{2+}$  release from the SR<sup>[45]</sup>. The inhibition of SR  $\text{Ca}^{2+}$  release through RyR2 was also observed in cardiac muscle, but the sensitivity to dantrolene was lower than in the skeletal muscle. Recently, it has been demonstrated that dantrolene can stabilize domain–domain interactions within the RyR complex<sup>[46]</sup>. Taken together, these data suggest that dantrolene might exert therapeutic effects in heart failure by preventing an abnormal SR  $\text{Ca}^{2+}$  leak, although this has not been investigated yet in experimental models<sup>[19]</sup>.

**Ryanoids** Ryanodine is a naturally-occurring plant alkaloid isolated from plants of the genus *Ryania* (Figure 1B). Because it binds with high affinity and specificity to its receptor in the SR, RyR have been named after this compound. Ryanodine is unusual in that it is a modulator of both gating and ion translocating properties of RyR2. The pharmacology of ryanodine has been described extensively in other literature reviews<sup>[20,47,48]</sup>, therefore we will focus on its action on RyR2 and its potential application as a drug for the treatment of cardiovascular disorders.

RyR2 possess both a high- and low-affinity binding site for ryanodine, which contributes to the concentration-dependent effects of ryanodine on the activity of RyR. At nanomolar concentrations, ryanodine increases the open



**Figure 1.** Molecular structures of RyR2 antagonists.

probability of RyR2 without affecting the rates of ion movement. At submicromolar concentrations, ryanodine increases RyR2 open probability to almost 100% and it induces a long-lasting subconductance state representing ~50% of the normal conductance level<sup>[47]</sup>. Finally, at high micromolar concentrations, ryanodine causes the channel to fully close, which accounts for an inhibitory effect on SR Ca<sup>2+</sup> release<sup>[49]</sup>.

Derivatives of ryanodine, collectively called ryanoids, display actions that do not conform to the canonical ryanodine characteristics. For example, some ryanoids can induce subconductance amplitudes far different from the half-

open state (ranging from 6%–75% of the maximum amplitude)<sup>[47]</sup>. The duration of the subconductance states also vary considerably among ryanoids. The unique features of certain ryanoids could be considered when developing ryanodine derivatives for heart failure therapy for which RyR2 specificity, low-level subconductance, and reversibility may be desirable characteristics.

**Local anesthetics** Several charged local anesthetics are known to inhibit RyR2 channels. These include both tertiary amines (eg procaine, tetracaine, and lidocaine), as well as quaternary amines (eg QX572 and QX314)<sup>[50]</sup>. Although procaine and tetracaine (Figure 1C) are both effective at low millimolar concentrations, procaine appears to be more selective for RyR2 compared to RyR1. Single channel studies have revealed that both drugs decrease the RyR2 open probability by stabilizing a closed conformational state<sup>[51]</sup>. Single channel studies of RyR2 have revealed 2 different modes of action of local anesthetics. Tetracaine and procaine decreased the channel open probability by stabilizing a closed state of the channel without affecting its unitary conductance<sup>[51]</sup>. In contrast, lidocaine (Figure 1D) and quaternary amines appear to induce voltage-dependent channel blockade, characterized by reduced conductance without changes in the open probability. This voltage-dependent inhibition is also observed in the presence of millimolar concentrations of procaine or tetracaine, in the presence of 2 μmol/L ryanodine<sup>[51]</sup>. Interestingly, tetracaine has been shown to prevent arrhythmogenic spontaneous SR Ca<sup>2+</sup> release events, presumably by reducing aberrant diastolic RyR2 openings<sup>[52]</sup>. Moreover, tetracaine also potentiates systolic Ca<sup>2+</sup> release due to enhanced diastolic SR Ca<sup>2+</sup> filling (due to decreased Ca<sup>2+</sup> leak from the SR)<sup>[53]</sup>. Thus, compounds such as tetracaine may have a therapeutic benefit in the prevention of cardiac arrhythmias and contractile dysfunction in heart failure.

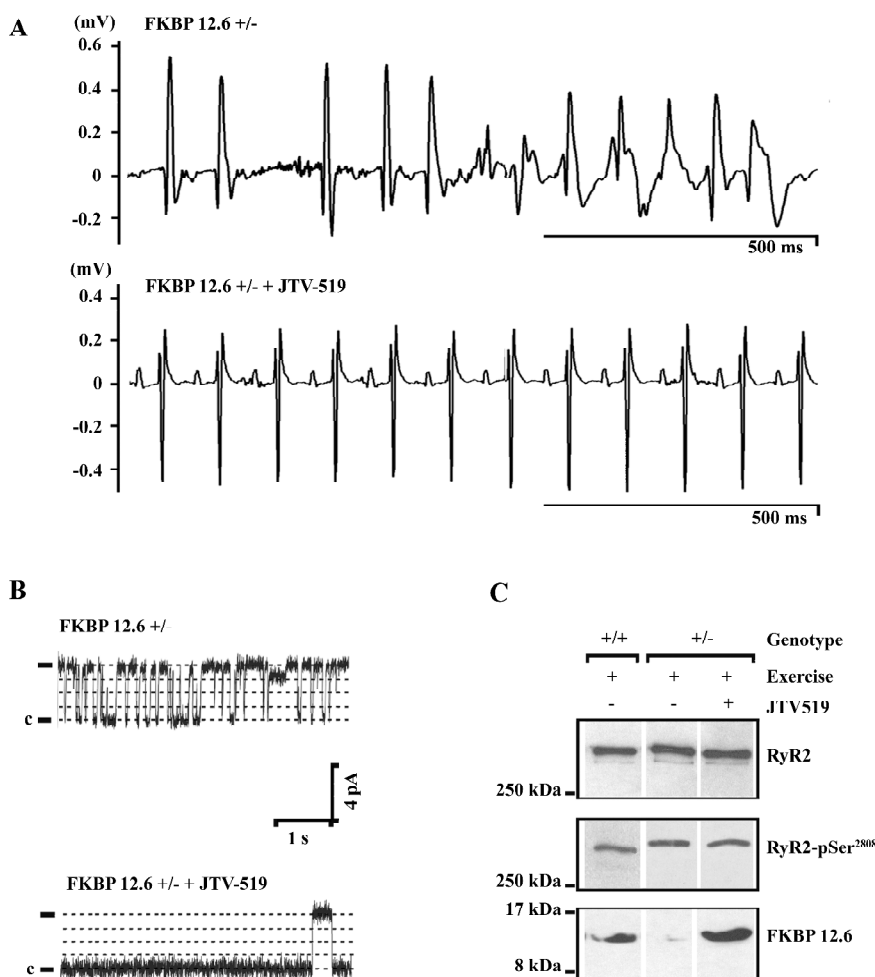
**1,4-Benzothiazepines** The pharmacological agents discussed earlier modulate RyR2 by directly altering channel gating or ion translocation. Recently, an additional mechanism for regulating RyR2 channels has been described<sup>[54,55]</sup>. It was shown that the 1,4-benzothiazepine derivative JTV519 (also known as K201, Figure 1E) stabilizes the interaction of RyR2 with the endogenous inhibitory subunit FKBP12.6<sup>[54-56]</sup>. The FK506-binding protein FKBP12.6 has previously been shown to stabilize a closed conformational state of the RyR2 channel, thereby decreasing the open probability<sup>[57]</sup>. In addition, JTV519 may enhance coupled gating between RyR2 channel complexes by increasing the binding of FKBP12.6<sup>[6]</sup>. Based on observations that FKBP12.6 binding to RyR2 is decreased in patients and animals with heart failure, the thera-

peutic role of JTV519 was assessed in disease models.

In animal models of heart failure and myocardial infarction, JTV519 has been demonstrated to improve contractile function and prevent the development of adverse left ventricular remodeling<sup>[54,56]</sup>. Because these therapeutic effects were not observed in FKBP12.6-deficient mice, it was concluded that these effects are dependent on the enhanced interaction of FKBP12.6 with RyR2<sup>[56]</sup>. Furthermore, it has been proposed that JTV519 may prevent cardiac arrhythmias that arise from delayed afterdepolarizations, initiated by a SR Ca<sup>2+</sup> leak through FKBP12.6-depleted RyR<sup>[58,59]</sup>. JTV519 prevented catecholaminergic ventricular tachycardias in FKBP12.6 haploinsufficient mice, but not in FKBP12.6-deficient mice, again indicating that the enhanced binding of FKBP12.6 to RyR2 constitutes the therapeutic mechanism of this 1,4-benzothiazepine derivative (Figure 2)<sup>[19,60]</sup>. Although these animal studies are very promising, it remains to be seen whether or not JTV519 becomes a useful clinical drug in the treatment of cardiac arrhythmias and heart failure.

## Conclusion

In this article, we provided a general overview of therapeutic approaches and pharmacological agents that are known to modulate RyR in the heart. Some of these compounds modulate channel gating, whereas others regulate ion translocation. The recent emergence of RyR2 as a critical defect in the pathogenesis of heart failure and triggered cardiac arrhythmias has spurred interest in developing novel therapeutic agents based on these channels. Although it is imperative that effective therapeutic agents do not interfere with systolic SR Ca<sup>2+</sup> release (as this would depress cardiac contractility), the inhibition of a diastolic SR Ca<sup>2+</sup> leak would be desirable as it likely prevents arrhythmias and enhances SR Ca<sup>2+</sup> loading (thus improving contractility). Most of the classic RyR2 modulating drugs, however, display unacceptable side effects or lack long-term efficacy. The recently described 1,4-benzothiazepine JTV519, however, acts via a different mechanism, namely by allosteric modification of



**Figure 2.** Pharmacological effects of JTV519. (A) FKBP12.6 haploinsufficient mice treated with JTV519 are protected from catecholamine-induced ventricular tachycardia. Representative ECG of an untreated FKBP 12.6 +/- mouse, and a JTV519-treated FKBP 12.6 +/- mouse. Mice were treated with 0.5 JTV519 mg·kg<sup>-1</sup>·h<sup>-1</sup> for 7 d with an implanted osmotic minipump. (B) Normalized RyR2 channel gating after treatment with JTV519. Representative single-channel tracings of FKBP 12.6 +/- mice, both untreated and after treatment with JTV519 (0.5 mg·kg<sup>-1</sup>·h<sup>-1</sup>). While haploinsufficiency of FKBP12.6 resulted in high probability for the open state of the channel, treatment with JTV519 decreased such probability significantly. (C) JTV519 enhances FKBP12.6 binding to RyR2, which is a novel pharmacological mechanism to modulate SR Ca<sup>2+</sup> release. Equivalent amounts of RyR2 were immunoprecipitated with an antibody against RyR2 (upper panel). Representative immunoblots show the amount of PKA phosphorylation of RyR2 at Ser2808, and the amount of FKBP12.6 bound to RyR2. Figure modified with permission from Wehrens *et al*<sup>[55]</sup>.

protein-protein interactions within the channel complex. Interestingly, this drug selectively targets diastolic SR Ca<sup>2+</sup> leaks without affecting systolic Ca<sup>2+</sup> release. Finally, these studies suggest that drugs that modify other subunits within the RyR2 macromolecular complex may also provide therapeutic benefits in patients with heart failure or arrhythmias.

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